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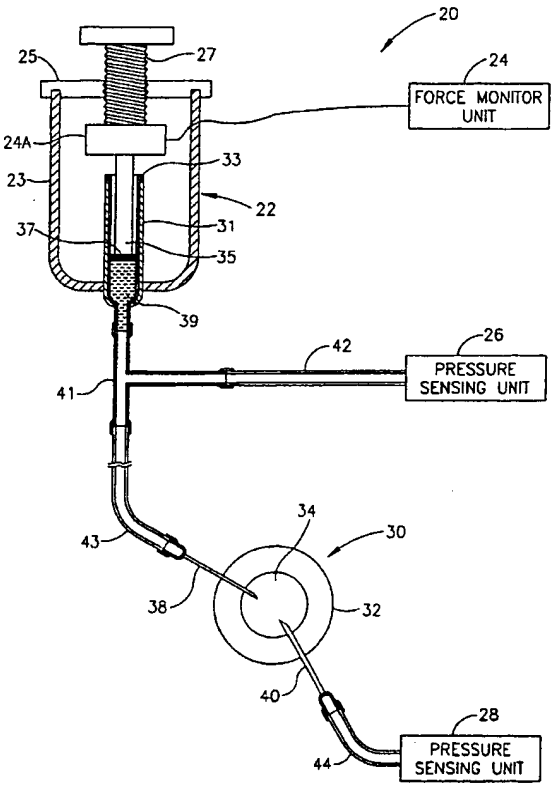
(54) Title: METHODS, DEVICES, AND PREPARATIONS FOR INTERVERTEBRAL DISC TREATMENT



(57) Abstract: A therapeutic method for treating mammalian intervertebral discs. A preparation of cross-linked collagen is injected under pressure into the intra-discal space. The intervertebral distance in injected discs is immediately increased by the treatment. At least some mechanical properties of the treated vertebral column are preserved or partially restored. The method may be used to relieve back pain in patients, to increase patient height and to stabilized the spinal column. The therapeutic method may result in at least a partial regeneration of the nucleus pulposus, and/or development of cartilaginous or fibrocartilaginous tissues or dense fibrous tissues.

WO 03/049669 A2

METHODS, DEVICES, AND PREPARATIONS FOR INTERVERTEBRAL DISC TREATMENT

FIELD OF THE INVENTION

The present invention relates generally to methods, devices, and preparations for treating intervertebral disc pathologies, and more specifically to preparations and implants including collagen, and cross-linked collagen and methods and devices for their introduction into mammalian intervertebral discs.

BACKGROUND OF THE INVENTION

Intervertebral discs are semi-elastic discs, which lie between the rigid bodies of adjacent vertebrae. Intervertebral discs form about one-fourth the length of the vertebral column.

Intervertebral discs are composed of two major anatomic zones, a peripheral part, the *annulus fibrosus* and a central part, the *nucleus pulposus*. The annulus fibrosus is composed of laminated fibrous tissue wrapped around the gelatinous nucleus pulposus. The parts of the vertebrae adjacent the intervertebral disc surfaces are called end plates. The nucleus pulposus of the Intervertebral discs are not vascularized and therefore depend on diffusion of nutrients through the endplates.

The annulus fibrosus is composed of laminae of fibrous tissue, in which collagen fibers are arranged in concentric layers or sheets also known as lamellae. The outer or peripheral layers of the annulus include type I collagen. In comparison, the inner annular layers lying next to the nucleus pulposus are referred to as the *transitional zone* and include fibrocartilaginous material. The collagen bundles in the laminae of the annulus fibrosus pass obliquely between adjacent vertebral bodies, and their inclination is reversed in alternate sheets. The collagen fibers' varying angles accommodate to all the angles of force that can be applied to the disc. The nucleus pulposus includes a lattice framework of collagen embedded in a highly hydrated gelatinous mass containing cartilage

annulus fibrosus, which may result, inter alia, in back pain and nerve root irritation (radiculopathy) due to the resulting nerve root or spinal cord compression.

The discs most commonly affected in intervertebral disc disease (IVD) or disc herniation (disc rupture) are those in spinal regions where a mobile part of the column joins a relatively immobile part, that is, the cervico-thoracic junction and the lumbo-sacral junction.

The role of the diseased intervertebral disc itself as a pain generator was not widely recognized until 1990's and many surgeons emphasize the nerve root compression component as the major factor to be addressed by surgical treatment. Nevertheless, the intervertebral disc as a pain generator is slowly gaining wider acceptance (*Spine* 20:665-669, 1995, *Spine* 20:1878-1883, 1995) and many people advocate removal of disc with or without herniation and nerve root compression (*Orthopaedics* 14:447-451, 1991, *Spine* 17: 831-833, 1992). Major forms of intervertebral disc disease or pathology include, inter alia, annular lamellar ruptures, disc herniation, ruptured posterior longitudinal ligament, annulus fibrosus (extruded disc material), and degeneration of the intervertebral disc.

The term degeneration of the intervertebral disc may imply an inevitable progression that is characteristic of wear-and-tear-associated conditions. Modern research on human tissue has, however, shown that this is not the case. The disruption of the micro-anatomy that is described as degeneration is an active process, which is probably regulated by locally produced cytokines. In disc degeneration, there is a disruption of the nucleus pulposus, including changes in the proportion and types of proteoglycans and collagens, a reduction in the number of chondrocytes, and the formation of permeative 'slit-like' spaces within the nucleus pulposus. Often, there is disruption of the collagen fiber arrays in the annulus fibrosus, traumatic damage to the disc end plate, and ingrowth of blood vessels and nerves into the nucleus pulposus. From our current understanding of the biology of connective tissues, it seems probable that alterations in the function of local cells are central to these events. (Tony J. Freemont, Christine LeMaitre, Alex Watkins and Judith A. Hoyland Histological

growth factor and vascular endothelial growth factor expression in disc herniation tissue: an immunohistochemical study.", Eur Spine J., 6, 63-69, 1997).

Lumbar spine decompression is a commonly performed procedure that is indicated for herniated nucleus pulposus. Current methods for lumbar spine decompression may be divided into open surgery and percutaneous surgical techniques. Open surgery techniques include lamina removal and fusion techniques. Percutaneous techniques include Laser Disc Decompression (PLDD) and electrosurgical spine treatment. Basically, surgery cannot repair the disc itself. What it can do is provide more room for the herniated disc to bulge in, thereby reducing pressure on the nerves which may reduce pain.

Surgical removal of an intervertebral disc or portions thereof may necessitate additional surgical procedures in cases where instability between spinal vertebrae is present. Spinal fusion or interbody fixation may be used in such cases. The advantages of interbody fixation include direct removal of the dysfunctional disc and preservation or restoration of the disc height. Maintenance of the disc height, is important to achieve significant increase in the neuroforamen volume.

One approach to stabilizing the vertebrae, termed spinal fusion, is to insert an interbody graft or implant into the space vacated by the degenerative disc. For example, in posterior lumbar interbody fusion (PLIF), two adjacent vertebral bodies are fused together by removing the affected disc and inserting an implant that would allow for bone to grow between the two vertebral bodies to bridge the gap left by the disc removal. A small amount of bone is grafted from other portions of the body, such as the hip, and packed into the implants. This allows the bone to grow through and around the implant, fusing the vertebral bodies and alleviating the pain.

One of the negative aspects of spinal fusion is that there is no movement between the two fused vertebra. The adjacent segments are extra-loaded when the spinal column bends. The result is that adjacent discs will degenerate faster.

compressed nerve root(s). According to the pathology, the laminectomy is performed on one side or bilaterally. Pressure is relieved by removal of the source of compression such as part of the herniated disc, a disc fragment, a tumor, or a bone spur.

Laminotomy is a less invasive, procedure which may be regarded as a refined version of laminectomy. In laminotomy only a small part of the lamina directly surrounding the affected disc is removed. There is growing evidence that laminotomy is superior. It is believed that the less bone that is removed, the more strong and stabile the remaining structure is. While performing those procedures will often relieve symptoms initially, there is a high incidence of subsequent complications, often worse than the original problem, because of the resulting spinal instability. Laminectomy as well as laminotomy may include an insertion of a space maintainer between the vertebrae.

US patent 6,283,968 discloses a posterior approach *laminectomy* procedure for placing a prosthesis within the intradiscal space between adjacent vertebrae.

In laminoplasty the back of the spine is exposed but instead of the bony structures being removed as done in laminectomy and laminotomy, they are being weakened and bent outwards thus opening the canal and providing more room for the spinal cord. The problem is how to stabilize the lamina in this new position.

Several techniques are used for lamina stabilization. One way of stabilizing the lamina is to take a bone graft from the *Illium* in the form of a rectangular plate of bone and wedge it in position to try and hold the lamina in its new, more open shape. This is generally effective but because it is not a firm arrangement, it can lead to some slippage and recurrent narrowing of the spinal canal. It also involves making a separate wound in the area of the *Illium* and taking a bone graft. Another technique uses a surgical implant device.

In percutaneus laser disc decompression (PLDD), a thin needle is inserted into the herniated disc at a forty-five degree angle, using Novocain for local anesthesia and X-ray guidance. An optical fiber is then inserted into the needle and laser beam is sent through the fiber, vaporizing a tiny portion of the disc

U.S patent No. 6,283,966 discloses instruments and methods for positioning a spinal implant within an *intervertebral* disc space between adjacent vertebrae.

U.S patent No. 6,258,125 discloses a method for regaining intervertebral space using an intervertebral allograft spacer.

U.S patent No. 6,240,926 discloses a method for regaining intervertebral space using hybrid materials composed of a biodegradable synthetic material such as bioactive glass or polymer foam and isolated intervertebral discs cells.

Deficiency of nucleus pulposus caused by degenerative changes or by surgical procedures of spine decompression may create secondary deterioration inside and around the disc.

The nucleus pulposus is sited within a chamber, the walls of which are made of the annulus fibrosus and the vertebral plates. The material, of which the nucleus pulposus is made, inhibits inflammation and vascular and neural proliferation within that chamber. Deficiency of nucleus pulposus material enhances freedom from that inhibition which may result in inflammation, and vascular and neural proliferation. This reaction may be further enhanced by macro-movements of the walls of the empty chamber.

Additionally, Lack of mechanical stabilization due to shrinking of the volume of the nucleus pulposus may result in gross-movements of the annulus fibrosus and eventually to the development of tears or fissures in the annulus fibrosus. Narrowing of the inter-vertebral space creates non-anatomical loads of the vertebral facet joints and may eventually lead to osteo-arthritic changes in those joints.

SUMMARY OF THE INVENTION

There is therefore provided , in accordance with an embodiment of the present invention, a method for treating a mammal with degenerative disc disease. the method includes injecting into at least one intervertebral disc of the mammal a volume of an injectable fluid including collagen cross-linked with a reducing sugar.

a biocompatible biodurable material to increase the distance between the two vertebrae attached to the disc(s).

There is further provided, in accordance with an embodiment of the present invention, a method for at least partially restoring or preserving at least one mechanical property of at least one degenerative disc in a patient. The method includes injecting into at least one intervertebral disc of the patient a volume of an injectable preparation including a biocompatible biodurable material to increase the distance between the two vertebrae attached to the at least one disc.

There is further provided, in accordance with an embodiment of the present invention, a method for treating a mammal with degenerative disc disease. The method includes pressure injecting into at least one intervertebral disc of the mammal a volume of an injectable preparation including collagen to reach a selected pressure level within the disc(s).

There is further provided, in accordance with an embodiment of the present invention, a method for inducing at least a partial regeneration of the nucleus pulposus, or development of cartilaginous tissue or fibrocartilaginous tissue or dense fibrous tissues in a degenerative mammalian intervertebral disc. The method includes injecting into the disc an injectable preparation including collagen cross-linked with a reducing sugar.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, in which like components are designated by like reference numerals, wherein:

Figs. 1A-1G are photomicrographs representing histological sections taken from pig intervertebral discs obtained in EXPERIMENT 1, including control discs, sham-operated discs and discs injected with ribose cross-linked collagen, in accordance with an embodiment of the methods of the present invention;

DETAILED DESCRIPTION OF THE INVENTION

Notation Used Throughout

The following notation is used throughout this document.

Term	Definition
IVD	Intervertebral disc disease
PLLD	Percutaneous laser disc decompression
PDN	Prosthetic disc nucleus
PLIF	posterior lumbar interbody fusion
VEGF	vascular endothelial growth factor
DDD	Degenerative disc disease

The present invention discloses a procedure for treating intervertebral discs having a nucleus pulposus deficiency or other types or discopathies or degeneracy by introducing a long acting biocompatible crossed-linked collagen into the intervertebral disc or another suitable injectable material that is biocompatible, and preferably also bio-durable. Preferably, the injected material or filler has water retaining capabilities. The cross-linked collagen may be introduced into the discal space which was occupied by the deficient nucleus pulposus. The cross-linked collagen or the other injectable filler material may mechanically stabilize the disc and the adjacent vertebrae.

In addition, the introduced cross-linked collagen may induce or promote the formation of fibrocartilage-like tissue within the chamber that may comprise fibrillar cross-linked collagen particles embedded within a cartilage-like or fibrous-like tissue, which may contribute to long term stabilization of biomechanical properties of the treated intervertebral to prevent discal deterioration processes.

The cross-linked collagen preparation may be introduced an the emptied nucleus pulposus space or into the intra-discal space of a degenerative intervertebral disc (without removing the nuclus pulposus). The procedure may be applied on painful intact annulus fibrosus discs using injectable cross-linked collagen preparations. Alternatively or additionally, the procedure may be performed on herniated discs having a damaged or fissured annulus fibrosus by utilizing compressed dry cross-linked collagen preparation.

removal via an annulus stab as is known in the art. These models are described in detail hereinafter in Experiments 1 and 3, respectively.

EXPERIMEMT 1

Pigs (*Sus domesticus*) weighting 50-60 kilograms were anesthetized. General anesthesia is established by a veterinary using fluoten, and an inter-tracheael tube. An anterior approach (specifically, a right retro pleural approach) to the thoracic vertebrae, was used. A sterile opening is made between the 6th and the 7th ribs. The ribs are spread apart and the lung with its wrappings is moved for obtaining a direct approach to the thoracic vertebrae. When the anterior approach is completed a retractor is applied and the breathing volume is decreased. A 22 gauge needle (22G) was introduced through the opening into the T6-T9 vertebrae region, and inserted through the annulus fibrosus of a intervertebral disc (selected from the discs of vertebrae T6, T7 or T8) into to the nucleus pulposus of the disc.

The insertion was performed at the right side of the disc. A high-pressure injector was connected to the 22G needle. Another 18 gauge needle (18G) was inserted into the nucleus pulposus of the same disc through the annulus fibrosus of the disc using a frontal disc penetration site. The 18G needle served as a draining outlet for washing out the contents of the nucleus pulposus. 10-20 milliliters of sterile saline was injected through the 22G needle at a pressure of about 10 atmospheres until the saline coming out of the 18G drainage needle was visually observed to be clear indicating that most of the material of the nucleus pulposus has been removed. A quantity of 0.5-2.0 milliliters of an injectable ribose crossed linked porcine collagen preparation was injected under pressure through the 22G needle until non–diluted collagen was visually observed to come out of the 18G needle used for drainage.

After the injection of the initial quantity of collagen is completed, the 18G drainage needle was withdrawn from the annulus fibrosus and another quantity of approximately 0.1-0.2 milliliters injectable cross-linked collagen is injected through the 22G needle until the collagen leaks out of the eyelet created by the 18G needle removal. The 22G needle is then withdrawn from the disc and a non-absorbable nylon stitch was made next to the treated vertebra for future

TABLE 1 below lists the details of the animals used in the histology examination experiments.

TABLE 1

Pig No.	Duration After Operation	Comments
V5	1 month	Histopathological evaluation completed.
V4	3 months	Histopathological evaluation completed.
V7	3 months	Histopathological evaluation completed.
V1	6 months	Histopathological evaluation completed.
V8	Not sacrificed yet	Pig reserved for follow-up at 12 months post operation.

A total of nine pigs participated in the study (labeled as pigs V1-V9). Histological evaluation was obtained from 4 out of 5 pigs which were sacrificed at 1, 3 and 6 months post operation (pigs V1, V4, V5, and V7). An additional pig (pig V8) was reserved for sacrificing at 12 months after the operation (histology results are not available yet for this animal). The four remaining pigs (pigs V2, V3, V6 and V9) were rejected due to infection or death during the operation that was not related to the administration of ribose cross-linked collagen.

The histology results are summarized in TABLE 2 below (and also presented in Figs. 1A-1G of the drawing figures).

The photo micrograph reveals the formation of an unfilled cavity 4 containing some debris.

Fig. 1C illustrates two different H&E stained histology cross section photomicrographs (the top photomicrograph at x40 magnification, and the bottom photomicrograph at x200 magnification) taken from a disc injected with ribose cross-linked collagen and harvested 1 month post-operatively (obtained from pig V5). The photomicrographs reveals that the injected ribose cross-linked collagen 5 is surrounded by hyaline cellular tissue. The intimate contact between the ribose cross-linked collagen 5 and the hyaline matrix of the nucleus pulposus 3 may be seen.

Fig. 1D illustrates an Alcian Blue stained histology cross section (at x200 magnification) from a disc injected with ribose cross-linked collagen and harvested 1 month post-operatively. The border 6 between the native tissues of the nucleus pulposus 3 and the injected Ribose cross-linked collagen 5 was basophilically stained, evidence to the absorption or in vivo deposition of proteoglycans onto the injected Ribose cross-linked collagen 5.

No inflammatory reactions (no inflammatory cells penetration), no granulation tissue, and no proliferation of blood vessels were seen in any of the specimens of animals harvested 1 month post-operatively.

Fig. 1E illustrates an H&E stained histology cross section (at x40 magnification) from a control sham-operated disc harvested 3 months post - operatively (obtained from pig V4). The empty space or cavity observed at 1 month could not be identified in this three month post operative section and the cavity was filled by high cellular hyaline tissue of the nucleus pulposus 3.

Fig. 1F illustrates an H&E stained histology cross section photomicrograph (at x40 magnification), taken from a disc injected with ribose cross-linked collagen and harvested 3 month post-operatively (obtained from pig V4). An intimate interaction of the ribose cross-linked collagen 5 with the adjacent hyaline tissue of the nucleus pulposus 3 is observed. No inflammatory reaction (no inflammatory cells penetration), no granulation tissue and no proliferation of blood vessels were seen in any of the specimens harvested three months post-operatively.

Injectable cross-linked bovine collagen and injectable cross-linked porcine collagen (100 microliters per injection site) were injected intra-cutaneousely into the ears of 20 New Zealand rabbits, using a 30 gauge (30G) needle. The needle penetrated the cartilage of the ear. Biopsies from the injected sites were obtained at one month post-injection and processed for histological examination.

All the injected sites for both porcine and bovine injectable cross-linked collagen could be physically identified at 1 month, 6 months and one year post-injection. No differences were observed in the tissue response to injectable cross-linked bovine collagen as compared to injectable cross-linked porcine collagen. Fibro-cartilage tissue was observed growing near and in the injected collagen based material. When cross-linked collagen was injected into and adjacent to the rabbit ear cartilage, chondroplasts were observed to populate the injected collagen matrix, forming cartilage structure in-between the cross-linked collagen particles. The general histological appearance was of a composite tissue consisting of cartilage reinforced with collagen particles. Some of these particles were also colonized by chondroblasts, and fibroblasts.

Reference is now made to Figs. 2A-2D which are photomicrographes illustrating the formation of cartilage tissue between particles of cross-linked collagen injected into the superficial part of rabbit ear cartilage as disclosed hereinabove. As can be seen in Figs. 2A-2D, chondroblasts migrated in between the injected cross-linked collagen material. In some sited indicated by the arrows, the condroblasts developed into islands of cartilage tissue. The biopsies of Figs. 2A-2D were obtained one month after injection.

The results illustrated in Figs. 2A-2D demonstrate the ability of the injected collagen preparations to promote the induction of cartilage and fibrocartillage formation in vivo. Therefore, when applied to intra-discal injection in humans, the therapeutic methods of the present invention may result in at least a partial regeneration of the nucleus pulposus, and/or development of cartilaginous or fibrocartilaginous tissues or dense fibrous tissues in the intra-discal space.

EXPERIMENTAL GROUP: This group included six pigs (pigs V10, V11, V12, V15, V16, and V17 of TABLE 3 below). At time zero (four months after the first operation), the pigs were anesthetized again and the operated degenerative discs were injected with 1.5 ± 0.2 milliliters of an injectable porcine ribose cross-linked collagen preparation (at a concentration of 35 milligram cross-linked collagen per milliliters of PBS). The injection was performed through a 22G spinal needle, using a pressure injection system capable of producing pressures of up to 10 atmospheres. The pressure injection system is described in detail hereinafter (See Fig. 3). The injection was done percutaneously using the image intensifier mobile Roentgen machine as disclosed hereinabove. The needle was inserted anterior and adjacent to the transverse processes of the vertebrae through the psoas muscle and the lateral side of the disc to the center of the disc while the animal was lying on its right side.

CONTROL GROUP: This group included five pigs that were operated to establish the DDD (pigs V13 ,V14, V18, V19, and V20 of TABLE 3 below). This group of animals was not injected with cross-linked collagen at zero time and served as a control.

SPINAL CANAL AND EPIDURAL INJECTION GROUP: Five pigs (pigs R10, R11, U3, U4, and U5 of TABLE 3 below) were tested to find out the reaction to an accidental leak of ribose cross-linked collagen into the spinal canal. In pig R10, under posterior open approach, 1.0 milliliter of ribose cross-linked collagen was injected into the intra-dural (spinal) space of the spinal canal at about L2-L3 and L3-L4 levels of one pig. In the remaining four pigs an epidural injection of approximately 1.0 milliliter ribose cross-linked collagen was performed. This was done in order to mimic the situation of a defect in the posterior wall of the annulus fibrosus leading to the extrusion of the material into the spinal canal. The animals were monitored for any development of neurological signs such as limping and incontinence. Six months after injection the animals will be sacrificed and the area of injection histologically examined.

Reference is now made to Fig. 3 which is a schematic part cross-sectional diagram illustrating the system used for intra-discal injecting of ribose cross-linked collagen and for monitoring the injection force and the pressure at

In the experiments of the present invention, the pressure sensing needle 40 was a standard stainless steel Luer lock type, 18 gauge (18G) needle. The pressure sensing needle 40 was connected through a reinforced high pressure flexible tube 44 to the pressure sensing unit 28 for measuring the intra-discal pressure (the pressure within the disc), before, during and after the injection of the ribose cross-linked collagen preparation 39.

The pressure sensing units 26 and 28 used in the experiments were model INDEFLATOR PLUS 20™ inflation devices including analog pressure gauge, commercially available from Guidant Advanced Cardiovascular systems, Inc., CA, USA. These devices have a measuring range of up to 20 atmospheres. Other suitable types of pressure sensing devices may also be used.

In the experiments, the force transducer 24A was attached between the forcing screw 27 and the end of the rod 35 for measuring the force acting on the rod 35. The force monitor unit 24 and the force transducer 24A were parts of force gauge unit commercially available as model FG-100kg force gauge from LUTRON Electronic Enterprise Co. Ltd. Taipei, Taiwan. The force gauge had a range of up to 100 kilograms of force with a nominal resolution of 50 grams of force.

In the experiments, the syringe 33 was loaded with ribose cross-linked collagen preparation containing ribose cross-linked porcine collagen at a concentration of 35 milligrams per milliliter of phosphate buffered saline (PBS). The forcing screw 27 was screwed in to exert pressure on the end of the rod 35 and to fill the T-junction 41, the tube 42 and the injection needle 38 with the injectable collagen preparation. Air was expelled by using PBS from the T-junction 41, the tube 42 and the needle 38 prior to insertion of the injecting needle 38 to enable pressure measurement by the pressure sensing unit 26 and to avoid the injection of air into the intra-discal space.

After the needles 38 and 40 were inserted into the disc 30 as disclosed in detail herein, the position of the end of the rubber piston 37 was recorded, and the forcing screw 27 was used to exert an increasing force on the end of the rod 35. The screw was advanced until a relatively stable pressure level of

ribose cross-linked collagen preparations having concentrations of up to 55 milligrams of cross-linked collagen per milliliter through needles having a gauge in the range of 22G - 31G without clogging of the needle, by using pressure levels at the outlet of the syringe 33 which did not exceed 10 atmospheres.

Thus, based on the known similarities between the structure, size, and mechanical properties of porcine and human intervertebral discs, it may be possible to inject the ribose cross-linked collagen preparations of the present in the concentration range of up to 55 milligrams/milliliters into the intra-discal space of intervertebral discs of mammals (including, but not limited to, porcine, canine and human discs). It is, however, noted that it may be possible to therapeutically inject ribose cross-linked collagen into mammalian intra-discal space at concentrations lower than 35 milligrams/milliliter or higher than 55 milligrams/milliliters, by, inter alia, changing the size of the injecting needle 38, changing the rheological properties of the injectable preparation, or by using other modifications of the methods and preparations of the present invention.

The preferred pressure range for intra-discal pressure (the pressure within the intervertebral space of the disc) may be within the range of approximately 0.5-10 atmospheres with the most preferred pressure range (as measured with the pressure sensing unit 28 during the injections) may be approximately 6-8 atmospheres.

Preferably, the intra-discal pressure should not exceed 10 atmospheres to avoid the possibility of disc rupture.

For porcine and human discs, the volume of ribose cross-linked collagen preparation which may be safely injected into the intra-discal space may be in the range of approximately 0.5 – 2.0 milliliters. The injectable volume may vary depending, inter alia, on the age and weight of the animal or patient, the size of the injected disc and position of the injected disc within the spinal column, and on the degree of intervertebral disc degeneration present. Other factors may, however also affect the injectable volume such as, for example, pathological changes in the mechanical parameters of the annulus fibrosus.

measurements of inter-vertebral disc functionality and movement was established. These measurements were used for assessing the efficacy of injectable cross-linked collagen in treating degenerative disc diseases in the animal models.

After sacrifice, intact spinal column segments including vertebrae L1 to L5 were retrieved from the sacrificed pig. The harvested segments included all the vertebral bony parts, joints, discs and ligaments without the attached muscles and skin. The column segments were deep frozen at -20°C. Before testing, the column segments were defrosted (Gleizes et al., 1998 has demonstrated that there is no difference in biomechanical results between fresh and thawed frozen spinal columns) and prepared for examination by connecting the spinal column segment at its ends with rigid plastic cement to the testing device as disclosed in detail hereinafter.

Measurement systems

In order to measure the effects of loading modes (bending and torsion) on segments of the vertebral column two separate systems were designed. Both systems were connected to the Instron 4502 machine. The INSTRON 4502 Automated Material Testing System is a dynamometer with a load cell commercially available from Instron Corp., MA, USA. Data of load and movements are synchronized and continually fed to a Personal computer (PC).

Reference is now made to Figs. 4A-4D which are photographs illustrating two configurations of the modified INSTRON Automated Material Testing System used in performing the biomechanical measurements including bending, flexing, and torsion experiments on pig spinal column segments from control animals and from animals having intervertebral discs injected with ribose cross-linked collagen, in accordance with an embodiment of the methods of the present invention.

Figs. 4A and 4B illustrate the positioning of the pig spinal column segment in the configuration of the INSTRON machine used for testing bending of the spinal column. Fig. 4B illustrates a part of Fig. 4A in detail.

The bending model system is based on previous bending measuring systems, described in the literature (Chiba M. et al., 1996; Marmelstein LE. et

When the INSTRON bridge 60 ascends, the arms 53 and 54 move one away from the other resulting in a moment which bends the vertebral column of the spinal column segment 55 in another direction, away from the bridge 60.

Two bending directions (flexion and extension) may be obtained. The applied force is measured directly by the load cell 50.

The movement measurement in the spinal column segment 55 is conducted using an extensiometer 62 (commercially available as catalogue number 2620-601, from Instron Corp., MA, USA). The arms of the extensiometer 62 are attached to the tested vertebrae (L2 and L4) in the movement direction. The upper arm of the extensiometer 62 is attached to vertebra L2 and the lower arm of the extensiometer 62 is attached to the vertebra L4. The acquired data is processed to generate a stress-strain curve representative of the force versus extension measurements (see the exemplary graphs of Figs.6A-6C hereinbelow).

Figs. 4C and 4D illustrate the positioning of the pig spinal column segment in the configuration of the modified INSTRON machine used for testing torsion of the spinal column. Fig. 4D illustrates a part of Fig. 4C in detail.

The torsion model is based on an arm 65 connected to a rotatable axle 67, to which the vertebral column segment 55 is fixedly attached. Two screws (not shown) fix the ends of a vertebrae column segment to a polyethylene cement casting, cast within the plastic cups 57 and 59 as disclosed in detail hereinafter. The cup 59 may be fixedly attached to a fixed non-rotatable holder 70 having a chuck at its end, while the cup 57 may be connected to a chuck at the end of the rotatable axle 67. The movable arm 65 is rigidly attached to the axle 67. The INSTRON machine controls the movement of the arm 65 by being coupled to one end of the arm 65 through a load cell 50. The force applied by the machine creates a torsion moment on the rotatable axle 67, and on the vertebrae column segment 55 attached thereto. The moment may be calculated by multiplying the arm length by the applied force as measured directly by the load cell 50. The rotary movement, given in degrees, is calculated from the movement of the INSTRON bridge 60 and from the length of the arm

57 and 59 are rigidly locked within the chucks at the ends of axle 67 and the holder 70, respectively. The position of the bridge 60 at the time of locking is (arbitrarily) set as zero.

The INSTRON machine was programmed to impose a load of up to 40 Newton on the part of the arm 65 connected to load cell 50, and to move the bridge 60 down at a rate of 180 millimeters/minute (Fig 4C). The other opposite part of the arm 65 has a constant load of 20 Newton acting thereon due to the weight attached thereto. Therefore, the moment was 5 Newton x meter. Once the maximum load level of 40 Newtons is developed (as detected by the load cell 50), the INSTRON machine reverses the direction of movement of the bridge 60 and the moment is released at the same bridge movement rate (180 millimeters/minute) until a 0.125 Newton x meter moment is reached, at which point the direction of movement of the bridge 60 is again reversed.

Five consecutive cycles were conducted for each test and the data was synchronically acquired and pooled by the PC, in order to present the results in a Stress-Strain graph.

Bending (flexion and extension) Examination: A prepared vertebral column segment (such as, for example, the spinal column segment 55 of Fig. 4A) was connected to the INSTRON machine, such that the sagital plane of the column segment 55 is at the movement plane of the arms 53 and 54 (See configuration illustrated in Fig. 4B). Bending of the spinal column segment 55 corresponding to the flexion-extension movement of the column was thus obtained. The two arms of the extensiometer 62 were fixed to the vertebrae in the movement's direction. The test started with a load of 35 Newton. The Instron's bridge 60 was programmed to rise at a speed of 180 millimeters/minute, for generating traction forces, until a force of 35 Newton was obtained. The bridge 60 then descended again at the same speed until a compression load force of 35 Newton was reached. Five consecutive cycles were conducted and the data was synchronically acquired and pooled by the PC. The results were presented as stress-strain curves.

linked collagen was injected in 2 out of 4 of the degenerated discs, as disclosed hereinabove.

Reference is now made to Figs. 5A-5D which are X-ray images of parts of the spinal vertebral column of Pig V10 illustrating the intervertebral spacing prior to and after intra-discal injection of ribose cross-linked collagen, in accordance with an embodiment of the methods of the present invention.

In pig V10, 1.5 milliliters of porcine ribose cross-linked collagen preparation was injected into each of the intervertebral discs at the L2-L3 level and the L3-L4 level, using the injection system illustrated in Fig. 3. The pressure measured at the outlet of the syringe 38 (by the pressure sensing unit 26 of Fig. 3) at the end of the injections at the L2-L3 level and the L3-L4 level was approximately 9 atmospheres.

Figs. 5A and 5B represent the X-ray images in the region of Vertebrae L2 and L3 prior to and immediately after the injection of ribose cross-linked collagen into the intra-discal space, respectively. In Fig. 5A which was taken after the insertion of the injecting needle into the intervertebral disc space of the disc between vertebrae L2 and L3 but before the injection of the cross-linked collagen, part of the injecting needle 38 may be seen entering the intra-discal space from above. The screws 60A and 60B are the cancellous screws inserted into the L2 and L3 vertebrae, respectively, as disclosed in detail hereinabove.

The X-ray photograph illustrated in Fig 5B was taken immediately after the injection of approximately 1.5 milliliters of ribose cross-linked collagen having a concentration of 35 milliliters of porcine ribose cross-linked collagen into the L2-L3 intra-discal space, without moving the pig or the imaging machine during the entire injection and imaging procedure. The same screws 60A and 60B may be seen in Fig. 5B.

Figs. 5C and 5D represent the X-ray images in the region of Vertebrae L3 and L4 prior to and immediately after the injection of ribose cross-linked collagen into the intra-discal space, respectively. In Fig. 5C which was taken after the insertion of the injecting needle into the intervertebral disc space of the disc between vertebrae L3 and L4 but before the injection of the cross-linked

Four days after ribose cross-linked collagen administration, pig V10 was sacrificed. The four degenerated discs and their facet joints were processed for histological examination as disclosed hereinabove.

Pigs V11-V20 were grouped into two subgroups. Pigs V11-V14 were destined for histopathological evaluation, while pigs V15-V20 were destined for biomechanical evaluation. The degenerative model was created in three discs in the V11-V14 pig subgroup, and in two discs the V15-V20 pig subgroup.

The histopathological and biomechanical evaluations will be completed by Q1 2003.

In addition to the 6 pigs (V15-V20) in subgroups B, segments of the lumbar vertebral columns of two additional pigs having approximately the same weight and age, which were not operated, were examined for biomechanics, and used as a second control group (non-operated, non-injected) for the degenerative disc model.

Five pigs, (pigs R10-R11 and U3-U5 of TABLE 3 above) are used for safety evaluation. Except for pig R10, which was injected in the intra dural (spinal) space, all the other pigs had an epidural injection. All pigs participating in EXPERIMENT 3 were generally in a very good condition, and displayed a perfect neurological performance. Neither limping nor urination problems were observed in any of the animals before sacrificing.

Exemplary results from Experiment 3

The mechanical tests were performed on intact L1-L5 vertebral column segments harvested ten (10) months after creating the model of disc degeneration in pig V19 and pig V16 of TABLE 3. In pig V16, disc L2-L3 and disc L3-L4 were injected with ribose cross-linked porcine collagen at the fourth month after the induction of the model DDD. The same mechanical tests were performed on a non-operated virgin vertebral column of a pig designated **control 1** (a non-operated pig having the same age and weight as the tested pigs). The three columns were tested using the INSTRON machine as disclosed in detail hereinabove. Five cycles of loading and unloading were performed for each of the three harvested columns at the physiological ranges of 40 Newtons X meter in flexion and extension, and torsion.

regions having a relatively large slope, in which regions the application of larger forces are needed to induce a displacement than in the middle region 80A of the curve 80.

Typically, in degenerative inter-vertebral discs, the range of displacement spanned by the (linear or nearly linear) middle part of the force versus displacement curve increases as compared to a comparable non-degenerative disc.

In Fig. 6D, the curve 110 represent the Force versus Displacement data for the five cycles of torsion measurements performed in the spinal column from the control1 pig. In Fig. 6E, the curve 112 represent the Force versus Displacement data for the five cycles of torsion measurements performed in the spinal column from the pig V16. In Fig. 6F, the curve 114 represent the Force versus Displacement data for the five cycles of torsion measurements performed in the spinal column from the pig V19. The displacement values represent the displacement in centimeters of the bridge 60 from an arbitrary zero point representing the position of the bridge 60 at the beginning of the test, as disclosed in detail hereinabove.

Turning to Fig. 6D, the curve 110 represents the displacement of the bridge 60 versus the force loading the arm 65 (to induce torsion in the lumbar spinal column segment being tested, as disclosed in detail hereinabove (in Fig. 6D the spinal column segment is from the control1 pig).

TABLE 6: Measurements of Torsion (range of displacement)

Pig designation	Bridge Displacement range (cm)
Control1	18.8
V16	18.4
V19	19.4

The measurement of the results of the torsion tests shown in TABLE 6 were performed on a parts the curves 110, 112 and 114 illustrated in Figs. 6D, 6E and 6F, respectively.

For each of the curves 110, 112 and 114, two points on the curve representing the reversal of bridge movement at the beginning and the end of the third torsion cycle were identified and marked on the curve. The values of

curve 80 of Fig. 6A. This value represents the total (non-linear) range of displacement under the experimental force used.

For the curves 90 and 100 of Figs. 6B and 6C, respectively the same measurement procedure as disclosed in detail for the curve 80 of Fig. 6A was used, and the values of the total extensiometer displacement range are given in TABLE 7.

In TABLE 8 below, the results of measurements of the middle nearly linear part of the uppermost and lowermost flexion curves for the pigs control1, V16 and V19, respectively, are shown. The range of displacement is given in centimeters.

TABLE 8:

Pig designation	Extensiometer Displacement range for Upper cycle portions (cm)	Extensiometer Displacement range for Lower cycle portions (cm)
Control1	0.56	0.54
V16	0.6	0.58
V19	0.66	0.62

The measurement of the results of the flexion and extension tests shown in TABLE 8 were performed on the curves 80, 90 and 100 illustrated in Figs. 6A, 6B and 6C, respectively.

For each of the curves 80, 90 and 100, the following procedure was used to determine the extensiometer displacement range for the upper and lower middle portions of the nearly linear parts of the experimental curves 80, 90 and 100. The procedure is explained for the particular example of curve 80 of Fig. 6A. Turning to Fig. 6A, the displacement range over which the force/displacement curve was nearly linear was visually estimated from all five curve portions 80AU in the upper part of the curve 80. Similarly, the displacement range over which the force/displacement curve was nearly linear was visually estimated from the four curve portions **80AL** in the lower part of the curve 80. The first cycle portion 87 was ignored since it represents part of the

From the clinical point of view these results show preservation of flexional and torsional mechanical properties of the column of the pigs which received the therapeutic injection of cross-linked collagen into the intra-discal space of model degenerate discs.

The flexion and torsion results shown above and illustrated in Figs. 6A-6F indicate that in accordance with an embodiment of the present invention, it may be possible to therapeutically preserve at least some of the mechanical properties of degenerated discs in a mammal by intra-discal injection of an injectable preparation of ribose cross-linked collagen.

EXPERIMENT 4

This experiment was performed to determine the relation between the pressure at the outlet of the syringe 33 (Fig. 3) and the pressure within the inner disc space of the disc 30 (Fig. 3) at the end of the injection of cross linked collagen.

Three virgin pigs (numbered as Pig 1, Pig 2 and Pig 3) having approximately the same weight as Pig V10 of EXPERIMENT 3 were sacrificed. The lumbar vertebral columns of all three were harvested as disclosed hereinabove and the discs at the L2-L3 and at the L3-L4 of each lumbar vertebral column segment were injected with 1.5 milliliters of porcine ribose cross-linked collagen injectable preparation having a concentration of 35 milligrams/milliliter of PBS. The system 20 (illustrated in Fig. 3) was used for performing the injection and pressure measurements as disclosed in detail hereinabove.

The pressure at the outlet of the syringe 33 was measured as disclosed hereinabove by the pressure sensing unit 26 (Fig. 3) at the end of the injection. The pressure in the inner disc space was measured as disclosed hereinabove by the pressure sensing unit 28 (Fig. 3) at the end of the injection.

The results of the pressure measurements are summarized in TABLE 9 below.

injection method may be used, inter alia, to improve or restore one or more mechanical properties of the spinal column including, but not limited to, the distance or spacing between vertebrae flanking the injected disc(s), the flexional and/or torsional mechanical properties of the spinal column, or other mechanical properties of the injected disc(s) or of the spinal column of the patient. Such therapy as disclosed herein may be used ,inter alia, to increase the height of the patient by increasing the distance between the vertebrae flanking the injected disc(s).

An advantage of the method of the present invention is that due to the volumes injected into the disc, the specific pressure range used for injection, and to the rheological properties of the injected material, the increase in intervertebral spacing and the improvement or restoration of spinal bio-mechanical properties is effected immediately after injecting the disc(s) and does not depend (at least for the short term effect of the injection) on the in-vivo induction or buildup of proteoglycans, or on the growth or restoration of nucleus pulposus tissue. It is, however noted that long term effects of the treatment method disclosed herein may also include the *in situ* deposition or production of proteoglycans within the intra-discal space or within the injected cross-linked collagen material injected into the treated disc, and may also include contributions due to longer term growth or restoration of nucleus pulposus tissue within the intra-discal space.

In applying the therapy in human patients, the volume and concentrations of the injected cross-linked collagen preparations may be similar to the experimental values used in pigs. The intra-discal pressure level within the injected human disc may preferably be in the range of 0.5-12 atmospheres, and more preferably in the range of 5 – 8 atmospheres and the injected volume used may be in the range of 0.5-2.5 milliliters, and the collagen concentration may be up to 55 milligrams of cross-linked collagen per milliliter of injectable preparation.

It is, however, noted that the above preferred values may be modified or adapted, depending, inter alia, on the patients age, the size and type of the disc(s) to be injected, and the species of the mammal which is being treated.

particulate collagen pellet washed several times in phosphate buffered saline (PBS) by repeated centrifugation and re-suspension.

The pellet spun down in the last washing may be resuspended in a 0.5-80% (w/v) D(-)Ribose solution in PBS. The volume of ribose solution used is approximately two times the volume of the spun down particulate collagen pellet. The resulting suspension is injected forcefully under pressure through an injecting device (such as a hollow stainless steel tube or needle) into 100% ethanol.

The stainless steel tube may be in the range of 14 gauge to 34 gauge, but other suitable tube sizes may also be used.

Preferably, the volume of the ethanol into which the above described collagen/ribose/PBS mixture is injected, and the exact concentration of the D(-)Ribose in PBS are selected such that after the injection is completed the final concentration of the D(-) ribose in the final mixture is approximately 1% (w/v), and the concentration of ethanol in the final mixture is 70% (v/v). It is noted that these concentrations are given by way of example only and other concentrations of D(-) ribose and/or ethanol may also be used, as disclosed in detail in international publication number WO 01/79342 A2. Furthermore, it may also be possible to use other sugar types for cross-linking the collagen as disclosed in detail in international publication number WO 01/79342 A2.

The injecting under pressure is performed in order to achieve proper dispersion of the small reconstituted fibrillar collagen particles in the cross-linking solution and to prevent aggregation of collagen particles due to the dehydrative action of the ethanol. The collagen suspension is then mixed and/or shaken for approximately 3 -14 days at a temperature in the range of 4°C-45°C for allowing the cross-linking of collagen to occur (other temperatures may also be used).

The resulting cross-linked collagen suspension is washed several times in phosphate buffered saline (PBS) by repeated centrifugation and re-suspension to remove the unreacted ribose and the ethanol. Then, the pellet is resuspended in PBS and allowed to equilibrate overnight for rehydration at 37°C (this equilibration step may be typically performed at temperatures in the range

limited to inducing bone formation, fibrosis or cartilage formation or the formation of other tissue types.

In accordance with other embodiments of the present invention, the injectable cross-linked collagen preparations used for treating intervertebral discs may be modified. For example, the injectable cross-linked collagen preparations may include additional substances, living cells, drugs, therapeutic materials or compounds or agents, and genetic material for gene therapy.

The living cells which may be added to the injectable cross-linked collagen preparation of the invention may include, inter alia, vertebrate chondrocytes, vertebrate stem cells, vertebrate progenitor cells, vertebrate fibroblasts, genetically engineered cells that are engineered to secrete matrix proteins, glycosaminoglicans, proteoglycans, morphogenic proteins, growth factors, transcription factors, anti-inflammatory agents such as proteins, hormones or peptides, or cells that have been engineered to express receptors to molecules such as proteins, glycosaminoglicans, proteoglycans, morphogenic proteins, growth factors, transcription factors, anti-inflammatory agents such as proteins, hormones, peptides, various different transcription factors, or cells that express any combination of the above described secreted substances and/or the above described receptors.

Other substances or compounds which may be included in the injectable cross-linked collagen preparation of the invention may include, inter alia, anaesthetic compounds or agents, anti-inflammatory compounds, agents or drugs, vertebrate growth factors proteins, glycosaminoglicans, proteoglycans, morphogenic proteins, anti-inflammatory agents such as proteins, hormones, peptides, and various transcription factors.

Other substances or compounds which may be included in the injectable cross-linked collagen preparation of the invention may include, inter alia, a nucleic acid, an oligonucleotide, ribonucleic acid, deoxyribonucleic acid, a chimeric DNA/RNA construct, DNA or RNA probes, anti-sense DNA, anti-sense RNA, a gene, a part of a gene, a composition including naturally or artificially produced oligonucleotides, a plasmid DNA, a cosmid DNA, or any other substance or compound containing nucleic acids or chemically modified nucleic

additional substances as disclosed hereinabove) in order to avoid contact of live cells with concentrated ethanol.

The advantages of using injectable cross-linked collagen preparations are several. The resistance of ribose cross-linked collagen to in-vivo biodegradation by naturally occurring collagenolytic enzymes, is much higher than in native collagen. It is therefore expected that the discs injected with the cross-linked collagen preparations may be less affected by such biodegradation while still being highly biocompatible.

Another advantage is that the collagen (cross-linked or non-cross-linked) may serve as a matrix for inducing and/or promoting the formation of cartilage or fibrocartilage in the disc. This is supported by the results of EXPERIMENT 2.

It is noted that while the ribose cross linked collagen used for intervertebral disc repair was derived from purified bovine and porcine atelopeptide collagen, many other types of collagen may be used in the disc repair and stabilization methods of the present invention, including but not limited to cross-linked and non-cross-linked collagen preparations obtained from other vertebrate species, including human collagen, cross-linked and non-cross-linked collagen preparations derived from recombinant collagen, or any other suitable types of genetically engineered or modified collagen.

It is further noted that although the ribose cross-linked collagen preparation was selected for use in disc repair due to its superior resistance to biological degradation in situ, it may also be possible to use non cross-linked collagen preparations in the same manner disclosed, though they may be degraded faster.

Additionally, it may be possible to use various mixtures of cross-linked and non-cross-linked collagen in the methods of intervertebral disc repair of the present invention, which may enable better control of the degradation resistance properties and other physical or chemical properties of the mixture. This may be particularly useful when live cells such as chondrocytes or other cells are added to the collagen preparation to promote tissue formation in situ (such as for example the formation of cartilage or fibrocartilage tissue). The mixing of

Cross-linked collagen implants for damaged or herniated intervertebral discs

It is noted that the advantages of the biocompatible collagen material may also be utilized in damaged, herniated or fissured intervertebral discs. In accordance with another preferred embodiment of the present invention, a collagen based implant is surgically introduced into the intervertebral disc after surgical removal of part of the material of the nucleus pulposus or of the entire nucleus pulposus. The removal of the nucleus pulposus may be performed using any surgical method known in the art for removal of part of or the entire nucleus pulposus.

The implant may comprises one or more pieces comprising dry collagenous material. Preferably, the collagenous implant or implants may comprise ribose cross-linked collagen due to its superior biodegradation resistance in vivo, but other types of collagenous materials may also be used. The collagenous implant(s) may act as space maintainers for stabilization of the damaged intervertebral disc and may also promote the formation of fibrocartilage tissue in situ which may further contribute to the long term bio-mechanical stabilization of the treated disc.

After the introduction of the dry collagen based implant or implants into the chamber previously occupied by the nucleus pulposus of the treated intervertebral disc, the dry implant(s) expand by rehydration. The size and shape of the implants may be adapted such that after dehydration and swelling they fill most of the space inside the treated disc after the surgical removal of the nucleus pulposus to provide mechanical support and stabilization of the intervertebral disc shape contributing to vertebral column stabilization and preventing further vertebral damage.

Due to the relatively small size of the dry swellable collagen based implants prior to swelling it may be possible to introduce the implants into the treated intervertebral disc through a small opening surgically made in the annulus fibrosus. Such methods for accessing the interior of the intervertebral disc for removal and/or introduction of materials are known in the art. For example, U.S. Patent 6,099,514 to Sharkey, incorporated herein by reference

opening due to their small volume in the dry or in the partially hydrated state. Additionally, in the case of implants including ribose cross-linked collagen an additional advantage is the high resistance to biodegradation in vivo.

A further advantage of the collagen based implants of the present invention is that even without the addition of isolated living chondrocytes, they may induce migration of the chondrocytes locally present in the disc and may encourage and induce fibrocartilage formation inside the disc as disclosed hereinabove and as demonstrated by the results of EXPERIMENT 2 above.

It is noted that while the method and preparations for treating intervertebral discs may be adapted to treat humans, the method and compositions of preparations may be applied similarly or with suitable modifications to treat intervertebral disc disease in other mammals or even in other vertebrates. For example, canine IVD is a known problem in certain dog breeds. The methods and injectable preparations disclosed may thus be applied to dogs or other pets or domestic or domesticated animals, as disclosed hereinabove.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made which are within the scope and spirit of the invention.

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CLAIMS

1. A method for treating a mammal with degenerative disc disease, the method comprises injecting into at least one intervertebral disc of said mammal a volume of an injectable fluid comprising collagen cross-linked with a reducing sugar.

2. The method according to claim 1 wherein said the concentration of said cross-linked collagen is in the range of 35-90 milligrams per milliliter of said injectable fluid.

3. The method according to claim 1 wherein said injectable fluid is injected into said at least one intervertebral disc to reach an intra-discal pressure level within a range of 0.5-12 atmospheres.

4. The method according to claim 1 wherein said injectable fluid is injected into said at least one intervertebral disc to reach an intra-discal pressure level within a range of 5-8 atmospheres.

5. The method according to claim 1 wherein said reducing sugar is D(-) ribose.

6. The method according to claim 1 wherein said collagen is reconstituted fibrillar atelopeptide collagen.

7. The method according to claim 1 wherein said mammal is a human patient and wherein said volume is within the range of 0.5 –2.5 milliliter.

8. The method according to claim 1 wherein said mammal is a human patient and wherein said volume is within the range of 1.0 –2.0 milliliter.

9. The method according to claim 1 wherein said injecting is performed by inserting a needle having a gauge in the range of 22G-31G through the annulus fibrosus of said at least one disc and injecting through said needle said volume into the internal space within said disc to reach a pressure in said internal space in the range of 0.5-12 atmospheres.

20. A method for therapeutically preserving at least one mechanical property of a degenerative mammalian intervertebral disc, the method comprises pressure injecting into said disc a volume of an injectable fluid comprising collagen cross-linked with a reducing sugar to reach a selected intra-discal pressure level within said at least one disc.

21. The method according to claim 20 wherein said selected intra-discal pressure level is within a range of 0.5-12 atmospheres.

22. The method according to claim 20 wherein said selected intra-discal pressure level is within a range of 5-8 atmospheres.

23. The method according to claim 20 wherein said reducing sugar is D(-) ribose.

24. The method according to claim 20 wherein said mammalian disc is a human intervertebral disc and wherein said volume is within the range of 0.5 – 2.5 milliliter.

25. The method according to claim 20 wherein said mammalian disc is a human intervertebral disc and wherein said volume is within the range of 1.0 – 2.0 milliliter.

26. The method according to claim 20 wherein said injecting is performed by inserting a needle having a gauge in the range of 22G-31G through the annulus fibrosus of said at least one disc and injecting through said needle said volume into the internal space within said disc to reach a pressure in said internal space in the range of 0.5-12 atmospheres.

27. The method according to claim 20 wherein said at least one mechanical property is selected from the spacing between the two vertebrae flanking said mammalian intervertebral disc, the flexional mechanical properties of said mammalian disc, and the torsional mechanical properties of said mammalian disc.

fibrosus of said disc and injecting said fluid through said needle into the internal space within said disc to reach an intra-discal pressure in said internal space in the range of 0.5-12 atmospheres.

37. The method according to claim 30 wherein the concentration of said cross-linked collagen is in the range of 35-90 milligrams per milliliter of said injectable fluid.

38. An injectable preparation for injecting into an intervertebral disc for inducing fibrocartilage formation in vivo, the preparation comprising an injectable fluid comprising particles of collagen cross-linked with a reducing sugar.

39. The injectable preparation according to claim 38 wherein said reducing sugar is D(-) ribose.

40. The injectable preparation according to claim 38 wherein the concentration of the cross-linked collagen is in the range of 35-90 milligrams per milliliter of said injectable preparation.

41. A method for increasing the height of a patient having a hydrodynamic disc dysfunction in at least one intervertebral disc, the method comprises injecting into said at least one disc a volume of an injectable fluid comprising collagen cross-linked with a reducing sugar to increase the distance between the two vertebrae attached to said at least one disc.

42. The method according to claim 41 wherein said increasing of height occurs during the injecting of said volume and is effective immediately after the injecting of said volume.

43. The method according to claim 41 wherein said injecting is performed to reach a selected intra-discal pressure level within a range of 0.5-12 atmospheres.

44. The method according to claim 41 wherein said injecting is performed to reach a selected intra-discal pressure level within a range of 5-8 atmospheres.

55. The method according to claim 50 wherein said volume is within the range of 1.0 –2.0 milliliter.

56. The method according to claim 50 wherein said injecting is performed by inserting a needle having a gauge in the range of 22G-31G through the annulus fibrosus of said at least one disc and injecting said volume through said needle into the internal space within said disc to reach an intra-discal pressure level in the range of 0.5-12 atmospheres.

57. The method according to claim 50 wherein the concentration of said cross-linked collagen is in the range of 35-90 milligrams per milliliter of said injectable fluid.

58. A method for relieving back pain resulting from degenerative disc disease in a patient, the method comprises injecting into at least one intervertebral disc of said patient a volume of an injectable preparation comprising a biocompatible biodurable material to increase the distance between the two vertebrae attached to said at least one disc.

59. The method according to claim 58 wherein said injecting comprises injecting into said at least one disc a volume of said injectable preparation sufficient to cause said increase of said distance immediately after said volume is injected.

60. The method according to claim 58 wherein said injecting comprises injecting into said at least one disc a volume of said injectable preparation sufficient to cause at least a partial relief of back pain immediately after said volume is injected.

61. The method according to claim 58 wherein said injectable preparation comprises a substance selected from collagen and hyaluronic acid.

62. The method according to claim 61 wherein said collagen is a cross-linked collagen and said hyaluronic acid is a cross-linked hyaluronic acid.

71. The method according to claim 65 wherein said injecting comprises injecting said injectable preparation to reach an intra-discal pressure said at least partial restoring immediately after said volume is injected.

72. The method according to claim 65 wherein said injecting comprises injecting said injectable preparation into said at least one disc to reach an intra-discal pressure level sufficient to cause at least a p[artial restoring of said at least one mechanical property immediately after said volume is injected.

73. A method for treating a mammal with degenerative disc disease, the method comprises pressure injecting into at least one intervertebral disc of said mammal a volume of an injectable preparation comprising collagen to reach a selected pressure level within said at least one disc.

74. The method according to claim 73 wherein said selected pressure level is within a range of 0.5-12 atmospheres.

75. The method according to claim 73 wherein said selected pressure level is within a range of 5-8 atmospheres.

76. The method according to claim 73 wherein said collagen is a cross-linked collagen.

77. The method according to claim 76 wherein said cross-linked collagen is cross-linked by a reducing sugar.

78. The method according to claim 77 wherein said reducing sugar is D(-) ribose.

79. The method according to claim 73 wherein said mammal is a human patient and wherein said volume is within the range of 0.5 –2.5 milliliter.

80. The method according to claim 73 wherein said mammal is a human patient and wherein said volume is within the range of 1.0 –2.0 milliliter.

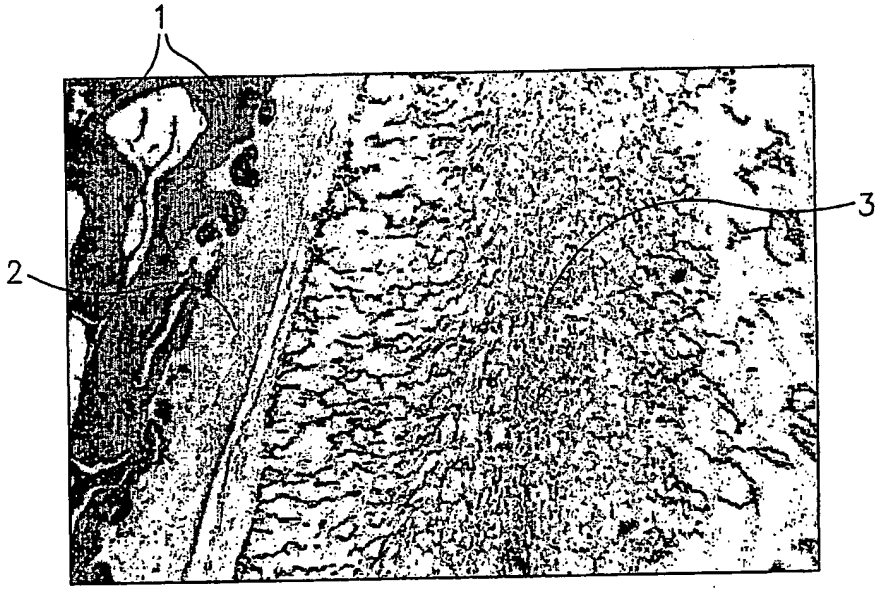

FIG.1A

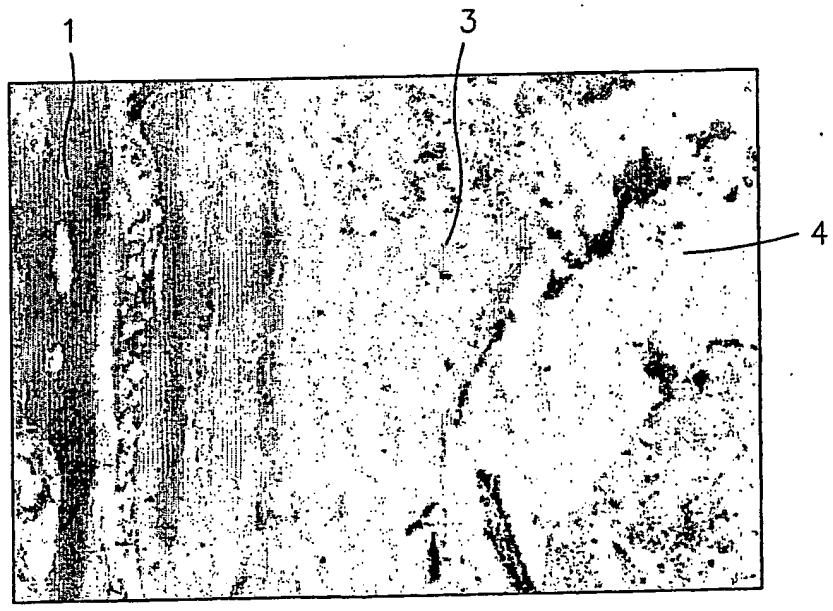

FIG.1B

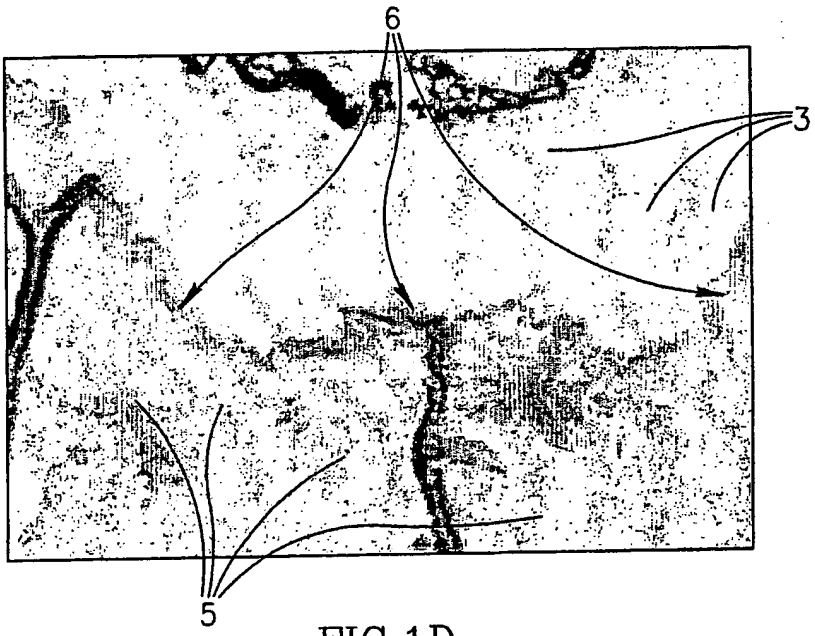

FIG.1D

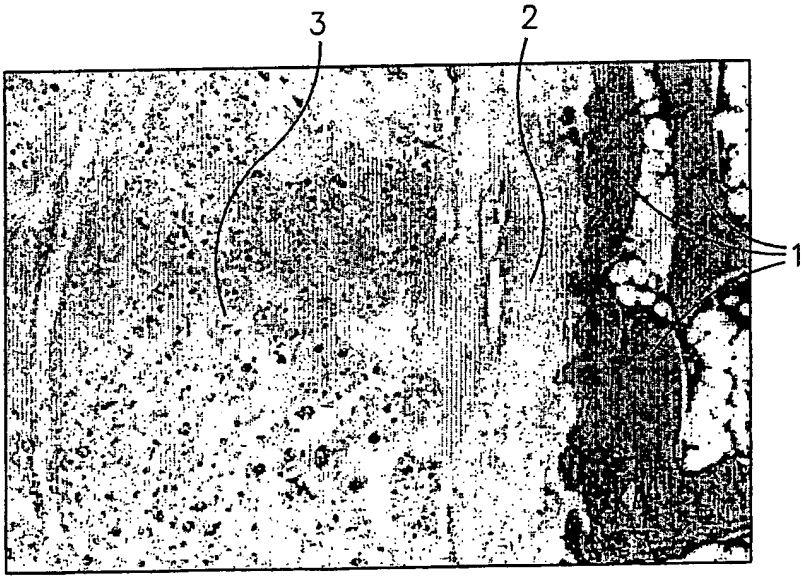

FIG.1E

FIG.2B

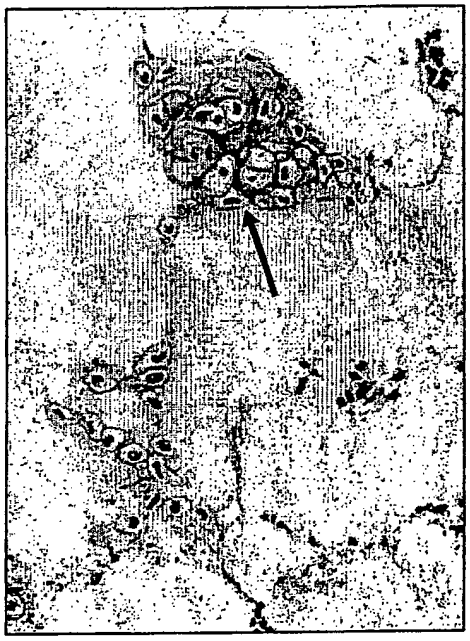

FIG.2D

FIG.2A

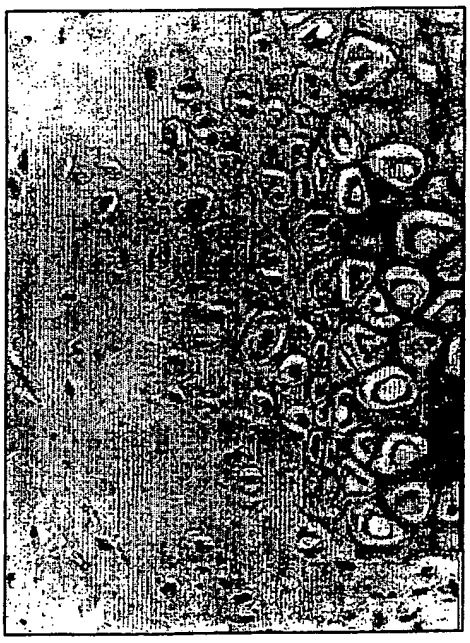

FIG.2C

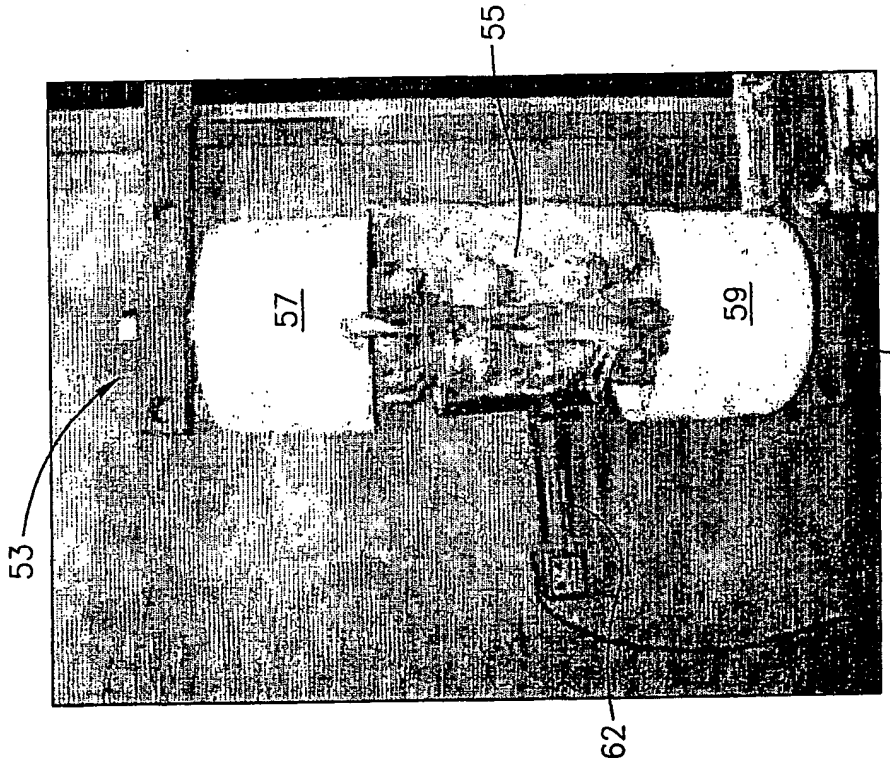

FIG.4B

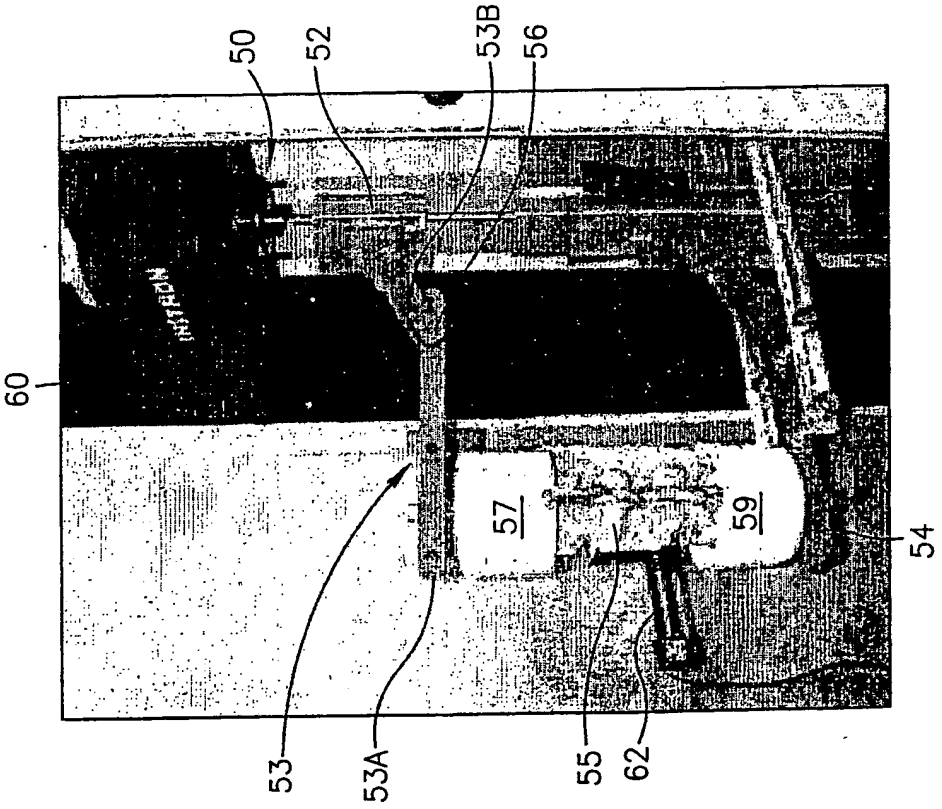

FIG.4A

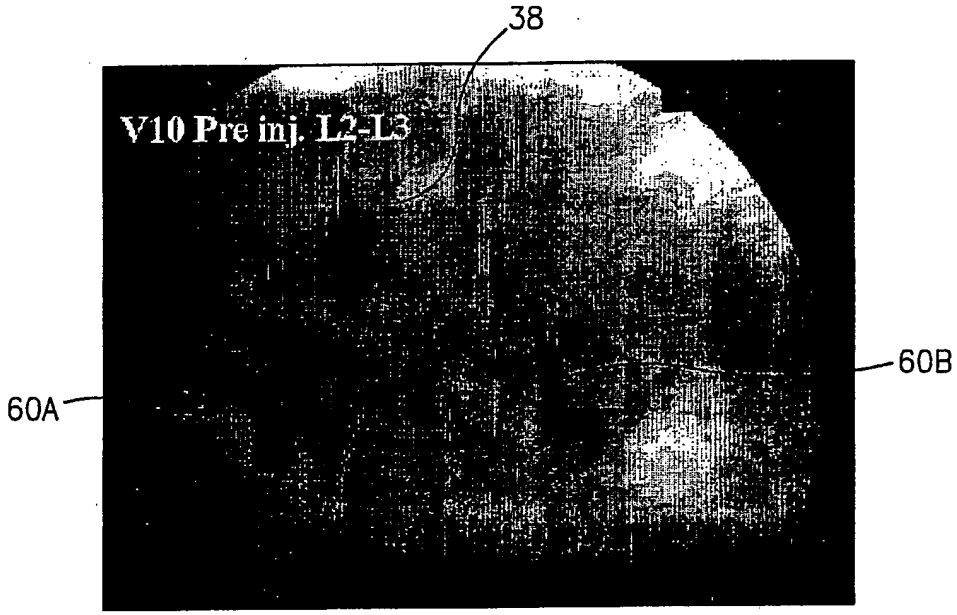

FIG.5A

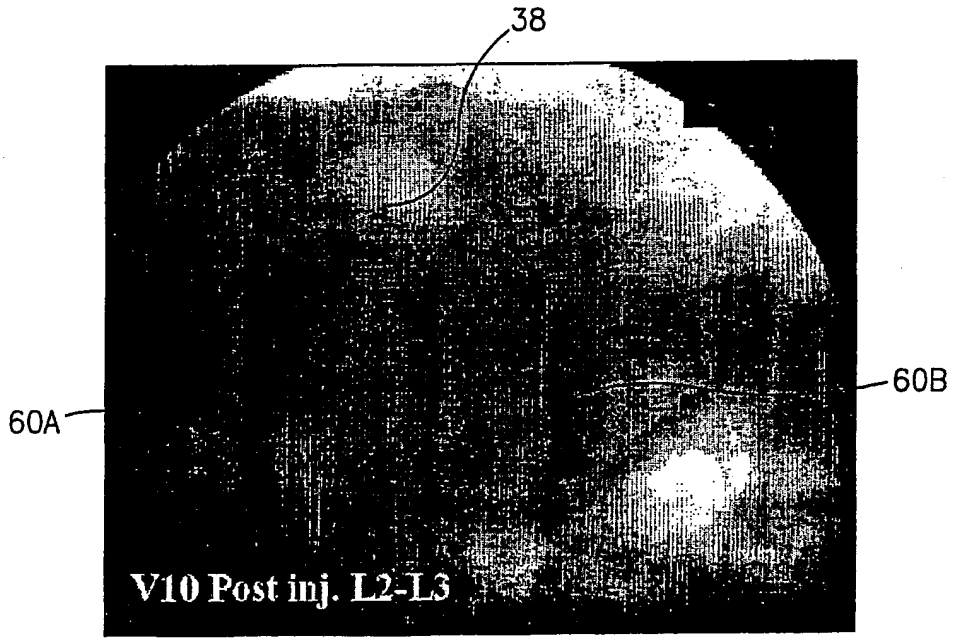

FIG.5B

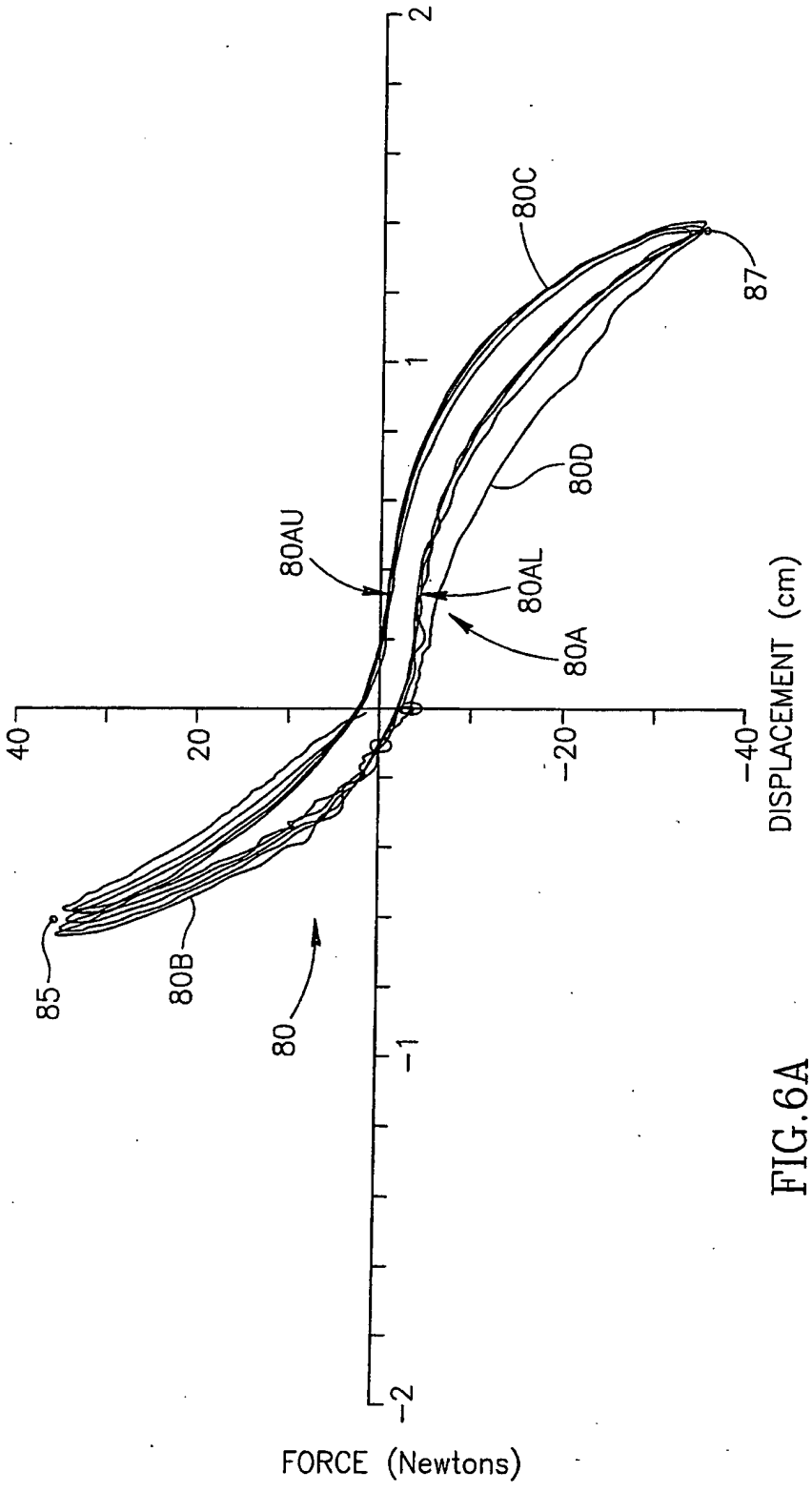
2
1
-1
-2
40
20
-20
-40
DISPLACEMENT (cm)
FORCE (Newtons)
80C
87
80D
80AU
80AL
80A
85
80B
80

FIG.6A

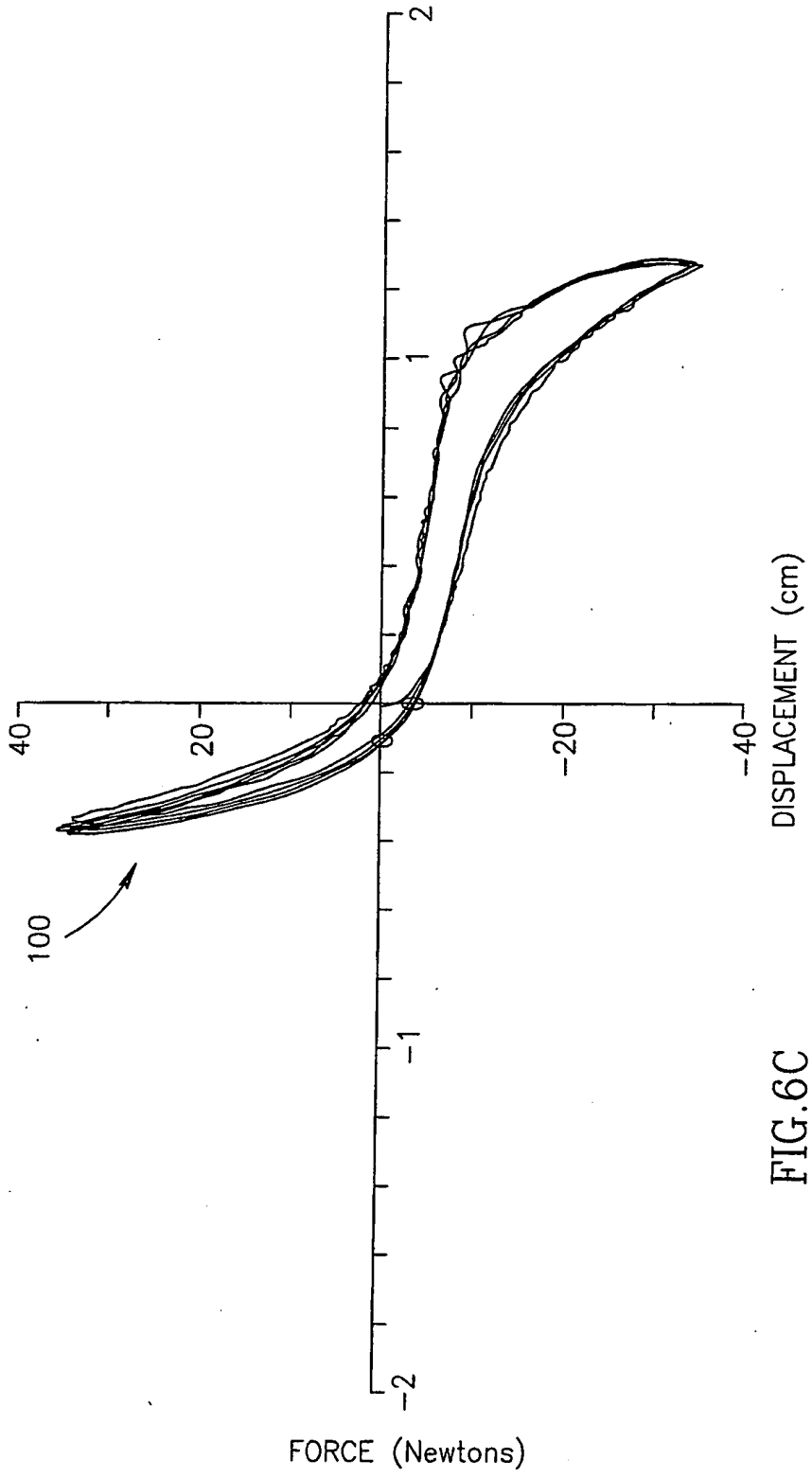

FIG.6C

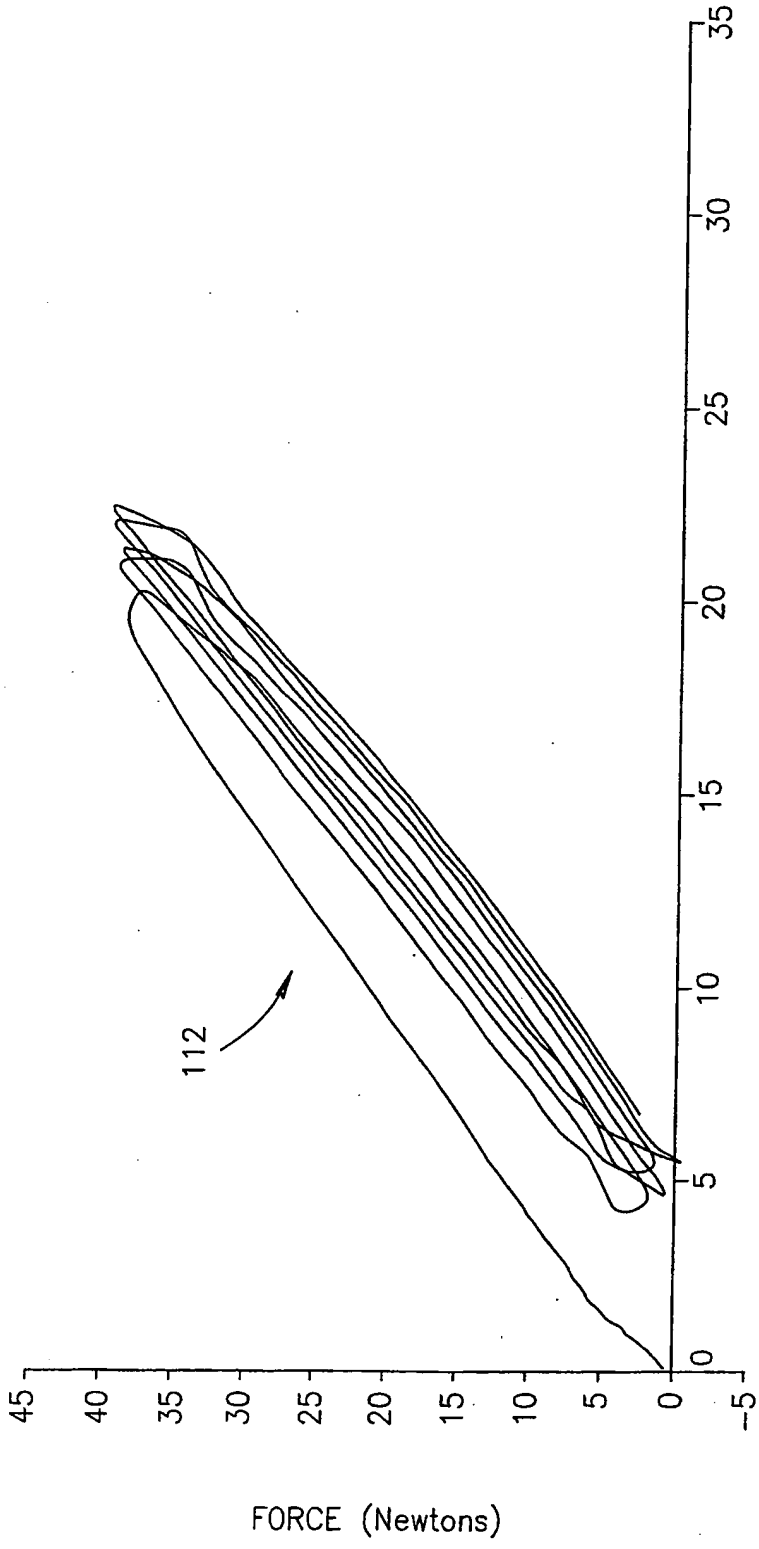
112
45
40
35
30
25
20
15
10
5
0
-5
FORCE (Newtons)
0
5
10
15
20
25
30
35
DISPLACEMENT (cm)

FIG.6E